Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate

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ABSTRACT

Background: Protein induces an increase in insulin concentrations when ingested in combination with carbohydrate. Increases in plasma insulin concentrations have been observed after the infusion of free amino acids. However, the insulinotropic properties of different amino acids or protein (hydrolysates) when co-ingested with carbohydrate have not been investigated.

Objective: The aim of this study was to define an amino acid and protein (hydrolysate) mixture with a maximal insulinotropic effect when co-ingested with carbohydrate.

Design: Eight healthy, nonobese male subjects visited our laboratory, after an overnight fast, on 10 occasions on which different beverage compositions were tested for 2 h. During those trials the subjects ingested 0.8 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate and 0.4 $g \cdot kg^{-1} \cdot h^{-1}$ of an amino acid and protein (hydrolysate) mixture.

Results: A strong initial increase in plasma glucose and insulin concentrations was observed in all trials, after which large differences in insulin response between drinks became apparent. After we expressed the insulin response as area under the curve during the second hour, ingestion of the drinks containing free leucine, phenylalanine, and arginine and the drinks with free leucine, phenylalanine, and wheat protein hydrolysate were followed by the largest insulin response (101% and 103% greater, respectively, than with the carbohydrate-only drink; $P < 0.05$).

Conclusions: Insulin responses are positively correlated with plasma leucine, phenylalanine, and tyrosine concentrations. A mixture of wheat protein hydrolysate, free leucine, phenylalanine, and carbohydrate can be applied as a nutritional supplement to strongly elevate insulin concentrations. *Am J Clin Nutr* 2000;72:96–105.

KEY WORDS Insulin secretion, amino acid supplementation, protein hydrolysates, leucine, arginine, phenylalanine, glutamine, healthy men

INTRODUCTION

The synergistically stimulating effect of the combined intake of carbohydrates and protein on plasma insulin concentrations was described for the first time in the 1960s (1, 2) and was confirmed later by Nuttall et al (3, 4). The insulinotropic effect of intravenous amino acid administration was also studied in the 1960s by Floyd et al (5–10). Infusion of several amino acids led to significant increases in plasma insulin. A mixture of 10 amino acids and equimolar quantities of arginine or leucine only were found to be the most potentiating. Floyd et al also observed a synergistic effect when glucose was administered intravenously with these amino acids. After different combinations of amino acids were investigated, the combined intravenous administration of arginine-leucine and arginine-phenylalanine, together with glucose, resulted in the largest increase in plasma insulin concentrations. Several in vitro studies using incubated β-cells of the pancreas showed strong insulinotropic effects of arginine, leucine, phenylalanine, and leucine in combination with glutamine (11–20).

A strong elevation of plasma insulin concentrations after the ingestion of carbohydrates in combination with a highly insulinotropic amino acid and protein mixture could be of experimental as well as practical importance. For example, in metabolic research such a mixture could be used as a tool to elevate insulin concentrations in vivo without intravenous insulin administration. In patients with type 2 diabetes, the mixture could be used as a means of evaluating the responsiveness of the pancreas. Although more research should be performed, such a mixture could possibly also be of use as a nutritional insulinotropic supplement during the early stages of declining insulin sensitivity in type 2 diabetes, potentially postponing patients' dependency on exogenous insulin administration. In sports nutrition, the addition of an insulinotropic amino acid and protein mixture to carbohydrate drinks could represent a means of increasing postexercise glycogen synthesis rates, as was shown by Zawadzki et al (21), and was investigated by us in another study (22).

Currently there is no literature available that provides clear insight into the type, combination, and quantity of free amino acids or protein sources that should be taken orally in combination

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TABLE 1
Composition of the 10 test drinks

Test drink	1	2	3	4	5	6	7	8	9	10
					g/L					
Arginine	—	57.1	19.0	14.3	—	—	—	—	—	9.50
Glutamine	—	—	—	14.3	—	—	—	—	—	—
Leucine	—	—	19.0	14.3	—	—	—	—	14.3	9.50
Phenylalanine	—	—	19.0	14.3	—	—	—	—	14.3	9.50
Whey hydrolysate	—	—	—	—	57.1	—	—	—	—	—
Pea hydrolysate	—	—	—	—	—	57.1	—	—	—	—
Wheat hydrolysate	—	—	—	—	—	—	57.1	—	28.6	28.6
Casein (milk protein)	—	—	—	—	—	—	—	57.1	—	—
Glucose	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1
Maltodextrin	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1
Sodium saccharinate	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Citric acid	1.80	1.80	1.80	1.80	1.80	1.80	1.801	1.80	1.80	1.80
Cream vanilla flavor	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

with carbohydrates to maximize the in vivo insulin response. The aim of this study was to define an amino acid and protein (hydrolysate) mixture with an optimal insulinotropic effect when added to a carbohydrate drink. A total of 10 drinks were tested in healthy subjects after an overnight fast to investigate the insulinotropic potential of several free amino acids, protein hydrolysates, and an intact protein. Because it was our aim also to investigate the efficiency of such an insulinotropic mixture in increasing postexercise muscle glycogen synthesis (22), similar amounts of carbohydrate and of the amino acid or protein (hydrolysate) mixture were administered, as used previously by Zawadzki et al (21). The choice of the free amino acid compositions tested in this study was based mainly on the outcome of studies by Floyd et al (5–10).

SUBJECTS AND METHODS

Subjects

Eight healthy, nonobese male subjects [$\bar{x} \pm$ SE age: 21 ± 0.4 y; weight: 73.9 ± 2.2 kg; height: 186 ± 2 cm; BMI (in kg/m^2): 21.4 ± 0.7] participated in this study. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. The study was approved by the Ethical Committee of the Academic Hospital Maastricht.

Experimental trials

Each subject participated in 10 trials, separated by ≥3 d, in which 10 different beverages were tested. All studies lasted 2 h, during which the subjects were seated and remained inactive. In the initial part of the study, test drinks 1 to 8 (*see* below) were tested. Beverages 9 and 10 were composed and tested within 3 wk after analysis of the acquired data on test drinks 1–8. In both parts of the study, beverages were provided in a random, double-blind fashion. All drinks were vanilla flavored so that the taste would be similar in all trials. The subjects were instructed to refrain from any sort of heavy physical labor and to keep their diets as constant as possible the day before the trials. The subjects had to fast for 12 h before reporting to the laboratory; during that period, the subjects were allowed to drink water or tea (without sugar).

Protocol

The subjects reported to the laboratory at 0830 after an overnight fast. A polytetrafluoroethylene catheter (Quick-Cath; Baxter Healthcare SA, Swinford, Ireland) was inserted into an antecubital vein and a resting blood sample was drawn at time zero. Immediately thereafter, the subjects drank an initial bolus (3.5 mL/kg) of a given test drink. Repeated boluses (3.5 mL/kg) were taken every 30 min for 90 min. Blood samples were drawn at 15-min intervals for measurement of plasma glucose and insulin concentrations. Amino acid concentrations were measured in blood samples taken at 0, 60, and 120 min.

Beverages

At 0, 30, 60, and 90 min, the subjects received a beverage volume of 3.5 mL/kg to ensure a given dose of 0.8 g carbohy-

TABLE 2
Amino acid composition of hydrolysates and intact casein protein

Amino acid	Whey	Pea	Wheat	Casein
		% by wt		
L-Alanine	4.7	3.8	1.8	3.1
L-Cysteine	1.2	0.4	0.9	0.4
L-Aspartate	5.4	4.4	0.2	3.7
L-Glutamate	9.1	7.4	3.2	11.2
L-Phenylalanine	2.4	3.2	4.8	5.4
L-Glycine	1.6	2.8	2.8	1.9
L-Histidine	1.6	1.7	1.6	3.2
L-Isoleucine	5.1	2.4	2.6	5.8
L-Lysine	8.4	5.9	—	8.3
L-Leucine	8.7	5.1	5.6	10.1
L-Methionine	1.3	0.6	1.1	3.0
L-Asparagine	4.4	3.8	1.9	3.7
L-Proline	5.9	2.8	12.3	10.5
L-Glutamine	7.4	6.6	29.0	11.2
L-Arginine	2.0	6.9	2.2	3.8
L-Serine	5.1	4.0	4.4	6.3
L-Threonine	6.6	2.8	2.0	4.6
L-Valine	4.5	2.7	3.0	7.4
L-Tryptophan	1.2	—	—	1.4
L-Tyrosine	2.3	2.6	2.5	5.8

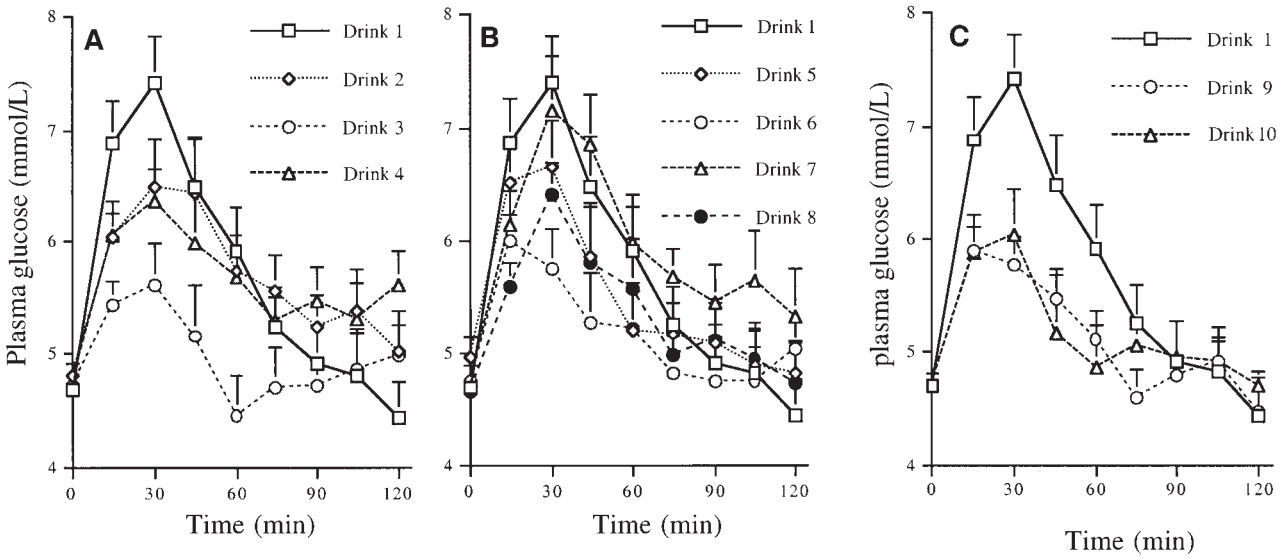

FIGURE 1. Mean (±SE) plasma glucose concentrations after ingestion of the control drink and drinks containing free amino acids (A), the control drink and drinks containing hydrolysates and an intact protein (B), and the control drink and drinks containing mixtures of hydrolysate and free amino acids (C). $n = 8$. For the exact compositions of the different drinks, *see* Tables 1 and 2.

drate/kg (50% as glucose and 50% as maltodextrin) and 0.4 g/kg of an amino acid and protein (hydrolysate) mixture every hour. The compositions of all test drinks are listed in **Table 1**. Glucose and maltodextrin were obtained from AVEBE (Veendam, Netherlands), crystalline amino acids were obtained from BUFA (Uitgeest, Netherlands), protein hydrolysates were prepared by Quest (Naarden, Netherlands), and sodium-casein was obtained from DMV (Veghel, Netherlands). Amino acid profiles of the protein hydrolysates and the intact protein tested were provided by the manufacturers and are listed in **Table 2**. All test drinks were uniformly flavored by adding 0.8 g sodium-saccharinate solution (25%, by wt), 3.6 g citric acid solution (50%, by wt), and 5 g of a cream vanilla flavor (Quest) for each 1-L drink.

Analysis

Blood (4 mL) was collected in EDTA-containing tubes and centrifuged at 1000 × *g* and 4°C for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −40°C. Glucose (Uni Kit III, 07367204; La Roche, Basel, Switzerland) was analyzed with the Cobas Fara semiautomatic analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Insulin RIA 100 kit; Pharmacia, Uppsala, Sweden). Plasma (200 μL) for amino acid analysis was deproteinized on ice with 50% (wt:vol) 5-sulfosalicylic acid and mixed, and the clear supernate obtained after centrifugation was stored at −80°C until analyzed. Amino acids were analyzed on an automated dedicated amino acid analyzer (LC5001; Biotronik, München,

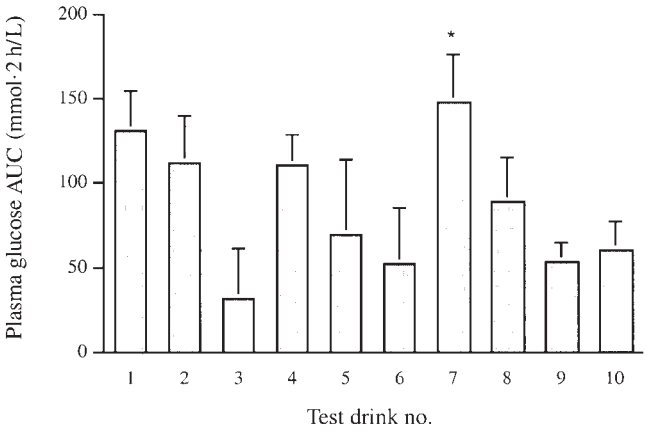

FIGURE 2. Mean (±SE) total 2-h plasma glucose responses (area under the curve; AUC) to each test drink. $n = 8$. *Significantly different from drink 3, $P < 0.05$. For the exact compositions of the different drinks, *see* Tables 1 and 2.

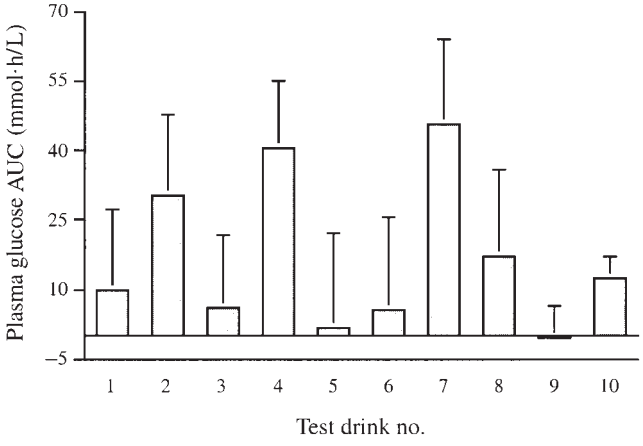

FIGURE 3. Mean (±SE) 2nd-h plasma glucose response (area under the curve; AUC) to each test drink. $n = 8$. For the exact compositions of the different drinks, *see* Tables 1 and 2.

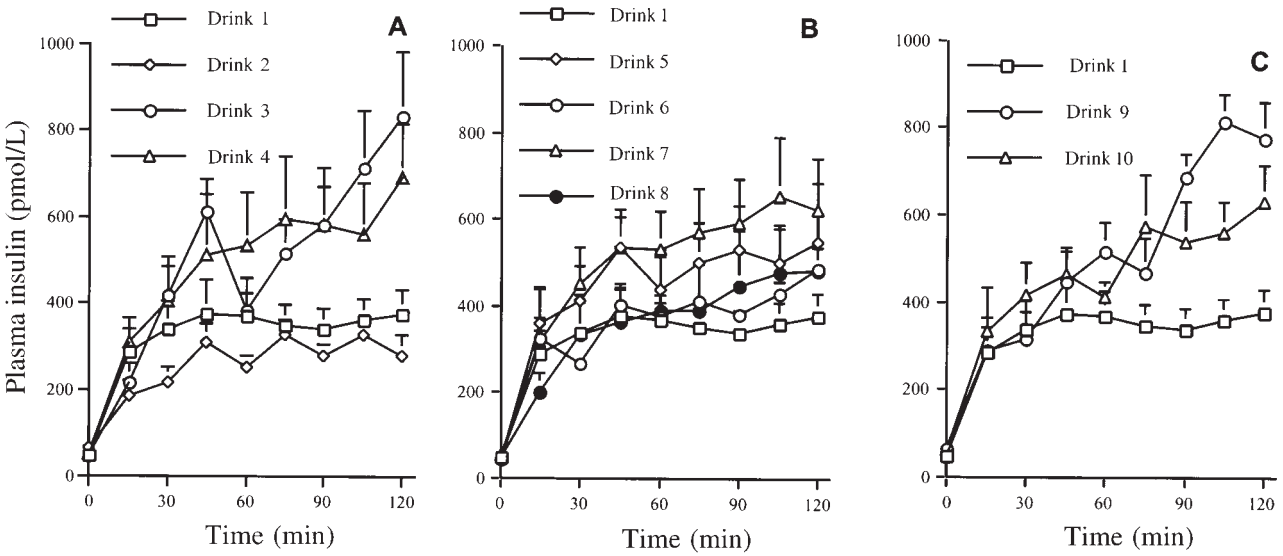

FIGURE 4. Mean (±SE) plasma insulin concentrations after ingestion of the control drink and drinks containing free amino acids (A), the control drink and drinks containing hydrolysates and an intact protein (B), or the control drink and drinks containing mixtures of hydrolysate and free amino acids. n = 8. For the exact compositions of the different drinks, *see* Tables 1 and 2. To convert from pmol/L to mU/L, divide by 7.25.

Germany) with use of a cationic exchange resin (type BTC2710; Biotronik), a gradient of lithiumcitrate elution buffers, and post-column derivatization with ninhydrin, all according to working recipes of the suppliers. The same procedures were performed to determine the amino acid composition of the protein (hydrolysates), except that a different amino acid analyzer was used (Pharmacia LKB Biotechnology, Roosendaal, Netherlands). Calibration curves of the amino acids were obtained by using commercial amino acid mixtures. Norvaline was used as internal standard and added to all plasma samples before deproteinization.

Questionnaires

After ingesting the beverage at 60 min and at the completion of each trial, the subjects were asked to fill out a questionnaire that contained questions about gastrointestinal discomfort and other complaints at that time. The presence of nausea, bloated feeling, belching, gastrointestinal cramping, vomiting, diarrhea, urge to defecate, urge to urinate, headache, and dizziness was scored on a 10-point scale (1, absent; 10, strongly present).

Statistics

All data are expressed as means ± SEMs (n = 8). The plasma glucose, insulin, and amino acid responses were calculated as area under the curve above baseline value (at 0 min). Statistical analysis of the data was performed by using a 2-factor (treatment and subjects) repeated-measures analysis of variance (ANOVA). Differences between drinks were tested for significance by using Tukey's post hoc test. In addition, simple regression analysis was performed to calculate correlations between the insulin response and the different plasma amino acid responses. Significance was set at $P < 0.05$.

RESULTS

Ingestion of all drinks resulted in an increase in plasma glucose concentrations during the first 30 min, after which concentrations decreased and stabilized during the second hour (**Figure 1**). After the glucose response was expressed as area under the curve (above baseline) during the entire 2-h trial, significant differences were found between drinks 3 and 7 (**Figure 2**: arginine, leucine, and phenylalanine and wheat protein hydrolysate, respectively). When we focused on the second hour, we found no significant differences in glucose response between the trials (**Figure 3**).

From baseline onward, plasma insulin concentrations strongly increased for the first 30–45 min. After this strong initial increase, insulin concentrations reached a plateau in trials 1 and 2, ie, the control (carbohydrate only) and arginine drinks, respectively (**Figure 4**A). Plasma insulin concentrations after the ingestion of drinks 5, 6, 7, and 8 increased more and leveled off between 45 and 60 min (Figure 4B). Insulin concentrations after ingestion of free amino acid–containing drinks 3, 4, 9, and 10 continued to increase during the last 30 min (Figure 4, A and C). After we expressed the insulin response as the area under the curve (above baseline) during the entire 2-h period, we found significant differences between drink 2 and drinks 3, 4, 7, and 9 (126%, 124%, 122%, and 105% greater than with drink 2, respectively) (**Figure 5**). Compared with the control trial, mean differences as large as 57%, 55%, 54%, and 42%, respectively were found (not significant). Because of the large intersubject variability during the first 45–60 min, caused by differences in gastric emptying and absorption rates, insulin responses were calculated over the second hour. During the second hour plasma insulin responses measured during the administration of drinks 3 and 9 were 2-fold higher than with the carbohydrate-only drink (101% and 103% greater, respectively) (**Figure 6**). Insulin responses after ingestion of drinks 3, 4, 7, and 9 were also significantly higher than after ingestion of the arginine drink (Figure 6). Insulin concentrations at 120 min were substantially higher after ingestion of drinks 3 and 9 than after ingestion of the control drink (122% and 106% greater, respectively).

Mean plasma amino acid concentrations at baseline, 60 min, and 120 min are reported in **Tables 3, 4,** and **5**. Plasma amino

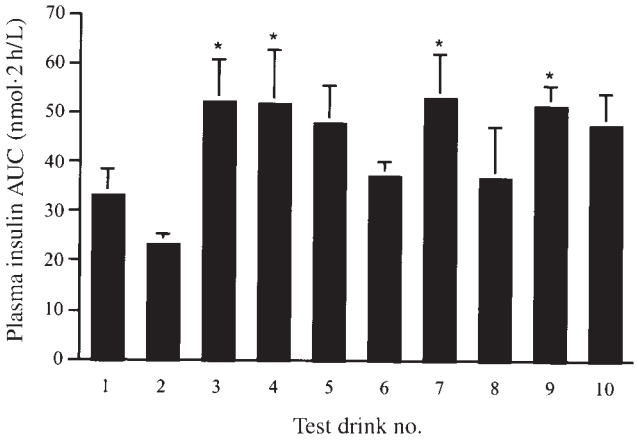

FIGURE 5. Mean (±SE) total 2-h plasma insulin response (area under the curve; AUC) to each test drink. $n = 8$. *Significantly different from drink 2, $P < 0.05$. For the exact compositions of the different drinks, *see* Tables 1 and 2.

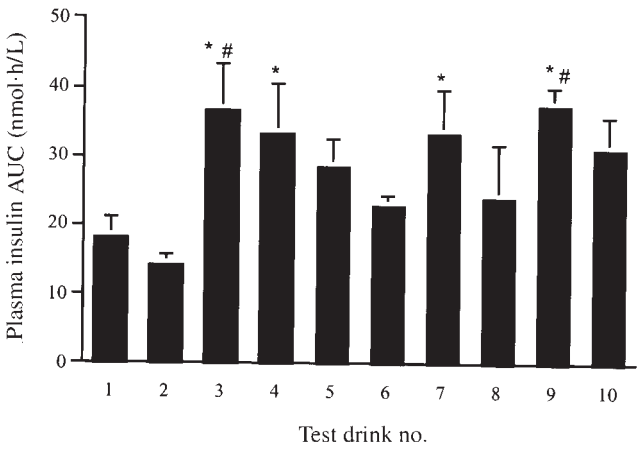

FIGURE 6. Mean (±SE) 2nd-h plasma insulin response (area under the curve; AUC) to each test drink. $n = 8$. #Significantly different from drink 1 (control), $P < 0.05$. *Significantly different from drink 2, $P < 0.05$. For the exact compositions of the different drinks, *see* Tables 1 and 2.

acid responses were calculated as area under the curve above baseline values. A complete overview of the plasma amino acid responses and the significant differences between the ingestion of the various mixtures is provided in **Table 6**. Only the findings most relevant to the aim of this study are discussed below. Plasma leucine, phenylalanine, and arginine concentrations increased significantly more after ingestion of drink 3 than after ingestion of all other drinks and in parallel showed one of the highest insulin responses. Ingestion of the other free amino acid–containing drinks (4, 9, and 10) also resulted in significantly higher plasma leucine and phenylalanine responses than did ingestion of the other drinks (1, 2, 5, 6, 7, and 8). Although the amount of free arginine administered in drink 2 was substantially larger than that provided in drinks 3, 4, and 10, the increase in plasma arginine concentrations was significantly lower after ingestion of drink 2 than after ingestion of drinks 3, 4, and 10. Addition of the

TABLE 3
Plasma amino acid concentrations after ingestion of drinks 1–4 containing amino acids and carbohydrate[1]

	Drink 1 (control)			Drink 2[2]			Drink 3[3]			Drink 4[4]		
	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min
	μmol/L											
Threonine	102 ± 7	90 ± 6	82 ± 6	108 ± 2	99 ± 6	87 ± 3	103 ± 5	85 ± 6	64 ± 4	93 ± 5	77 ± 5	61 ± 4
Serine	91 ± 5	81 ± 6	70 ± 5	98 ± 6	88 ± 7	75 ± 5	90 ± 8	73 ± 7	55 ± 6	87 ± 7	72 ± 6	57 ± 5
Asparagine	52 ± 4	46 ± 4	42 ± 3	49 ± 2	44 ± 4	38 ± 2	50 ± 3	40 ± 3	31 ± 2	48 ± 3	39 ± 3	31 ± 3
Glutamate	78 ± 10	83 ± 9	76 ± 10	124 ± 11	111 ± 9	127 ± 5	110 ± 16	85 ± 10	73 ± 10	115 ± 9	76 ± 8	80 ± 8
Glutamine	667 ± 32	624 ± 21	600 ± 28	631 ± 29	597 ± 32	566 ± 11	578 ± 25	601 ± 31	556 ± 14	559 ± 28	642 ± 29	616 ± 30
Proline	147 ± 13	134 ± 14	128 ± 15	162 ± 18	164 ± 25	154 ± 19	174 ± 21	144 ± 18	124 ± 18	160 ± 21	128 ± 20	112 ± 19
Glycine	217 ± 16	201 ± 15	192 ± 14	228 ± 11	203 ± 9	198 ± 11	221 ± 12	175 ± 10	142 ± 8	218 ± 15	174 ± 14	148 ± 15
Alanine	291 ± 25	302 ± 21	306 ± 18	334 ± 34	314 ± 32	350 ± 20	324 ± 31	297 ± 23	271 ± 17	296 ± 24	296 ± 20	284 ± 18
Citrulline	28 ± 2	16 ± 1	12 ± 1	30 ± 1	26 ± 2	20 ± 2	28 ± 2	30 ± 2	29 ± 2	28 ± 1	33 ± 2	40 ± 3
α-Aminobutyrate	21 ± 2	20 ± 2	19 ± 1	21 ± 3	20 ± 3	18 ± 3	18 ± 3	17 ± 3	15 ± 1	21 ± 2	19 ± 2	16 ± 1
Valine	224 ± 7	195 ± 7	169 ± 5	247 ± 5	219 ± 6	185 ± 2	236 ± 9	194 ± 7	118 ± 5	218 ± 6	177 ± 6	111 ± 5
Methionine	24 ± 1	20 ± 1	16 ± 1	23 ± 1	20 ± 1	15 ± 1	22 ± 1	19 ± 1	10 ± 0	21 ± 1	16 ± 1	9 ± 1
Isoleucine	64 ± 1	48 ± 2	33 ± 2	67 ± 4	53 ± 3	30 ± 2	62 ± 3	40 ± 2	11 ± 1	63 ± 2	41 ± 3	11 ± 1
Leucine	121 ± 4	90 ± 6	67 ± 4	131 ± 3	108 ± 6	73 ± 3	116 ± 5	568 ± 48	804 ± 32	117 ± 4	502 ± 37	584 ± 67
Tyrosine	58 ± 3	51 ± 4	44 ± 3	61 ± 3	52 ± 4	42 ± 3	55 ± 3	99 ± 8	131 ± 12	50 ± 2	96 ± 7	167 ± 44
Phenylalanine	57 ± 3	47 ± 4	41 ± 3	67 ± 2	57 ± 3	49 ± 2	61 ± 2	407 ± 32	681 ± 22	60 ± 2	328 ± 25	473 ± 69
Tryptophan	44 ± 3	39 ± 3	33 ± 3	45 ± 2	43 ± 3	37 ± 2	40 ± 2	34 ± 2	23 ± 2	36 ± 2	30 ± 1	37 ± 17
Ornithine	47 ± 3	42 ± 5	36 ± 3	53 ± 3	87 ± 8	163 ± 15	54 ± 5	143 ± 11	260 ± 24	50 ± 6	125 ± 11	197 ± 21
Lysine	155 ± 9	143 ± 10	133 ± 8	168 ± 6	164 ± 12	153 ± 9	161 ± 7	181 ± 11	158 ± 8	143 ± 7	149 ± 8	124 ± 15
Histidine	73 ± 2	67 ± 1	63 ± 2	79 ± 3	72 ± 4	65 ± 3	72 ± 2	62 ± 3	47 ± 2	70 ± 2	61 ± 3	52 ± 2
Arginine	76 ± 5	66 ± 4	58 ± 4	83 ± 2	164 ± 8	286 ± 19	79 ± 3	371 ± 25	538 ± 29	75 ± 3	299 ± 22	410 ± 24

[1] $\bar{x}$ ± SEM; $n = 8$. For compositions of drinks 1–4, *see* Tables 1 and 2.
[2] Arginine.
[3] Leucine, phenylalanine, and arginine.
[4] Leucine, phenylalinine, arginine, and glutamine.

TABLE 4
Plasma amino acid concentrations after ingestion of drinks 5–8 containing protein (hydrolysate) and carbohydrate[1]

	5 (whey)			6 (pea)			7 (wheat)			8 (casein)		
	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min
						μmol/L						
Threonine	118 ± 7	217 ± 12	258 ± 16	113 ± 6	166 ± 9	185 ± 8	109 ± 7	138 ± 12	136 ± 12	113 ± 6	126 ± 6	125 ± 6
Serine	101 ± 7	150 ± 11	155 ± 13	101 ± 8	141 ± 9	153 ± 9	98 ± 7	134 ± 8	136 ± 12	98 ± 10	109 ± 11	102 ± 10
Asparagine	46 ± 3	81 ± 6	91 ± 7	54 ± 4	98 ± 8	115 ± 8	52 ± 2	62 ± 4	58 ± 4	50 ± 2	57 ± 3	57 ± 4
Glutamate	56 ± 5	62 ± 5	55 ± 4	63 ± 9	60 ± 9	69 ± 10	104 ± 10	86 ± 8	72 ± 11	49 ± 11	42 ± 6	43 ± 8
Glutamine	756 ± 25	815 ± 29	816 ± 36	663 ± 25	725 ± 30	727 ± 32	628 ± 23	754 ± 21	769 ± 22	721 ± 29	722 ± 23	700 ± 27
Proline	174 ± 21	265 ± 24	306 ± 25	173 ± 20	226 ± 25	252 ± 22	181 ± 20	327 ± 24	393 ± 31	175 ± 19	220 ± 23	245 ± 20
Glycine	244 ± 16	239 ± 14	254 ± 20	238 ± 8	285 ± 12	307 ± 14	231 ± 11	260 ± 14	273 ± 16	237 ± 14	229 ± 14	224 ± 15
Alanine	305 ± 25	462 ± 17	489 ± 14	326 ± 30	457 ± 26	488 ± 22	317 ± 19	425 ± 25	438 ± 17	353 ± 30	382 ± 19	396 ± 14
Citrulline	34 ± 2	30 ± 2	33 ± 2	32 ± 2	27 ± 2	30 ± 2	32 ± 2	37 ± 3	42 ± 4	31 ± 2	26 ± 2	25 ± 2
α-Aminobutyrate	30 ± 3	34 ± 5	36 ± 4	18 ± 2	19 ± 2	18 ± 2	19 ± 2	21 ± 2	19 ± 2	17 ± 2	17 ± 2	16 ± 2
Valine	254 ± 10	366 ± 11	403 ± 8	241 ± 3	312 ± 7	344 ± 7	243 ± 6	293 ± 8	294 ± 8	248 ± 8	268 ± 8	262 ± 6
Methionine	25 ± 1	40 ± 1	47 ± 2	24 ± 1	23 ± 1	18 ± 1	24 ± 1	31 ± 1	30 ± 1	24 ± 1	27 ± 1	27 ± 1
Isoleucine	69 ± 4	155 ± 5	185 ± 7	63 ± 2	107 ± 4	121 ± 4	70 ± 2	98 ± 3	95 ± 5	72 ± 3	81 ± 3	79 ± 3
Leucine	131 ± 5	253 ± 6	294 ± 8	122 ± 3	191 ± 6	213 ± 7	125 ± 4	181 ± 7	188 ± 9	123 ± 4	134 ± 4	127 ± 4
Tyrosine	59 ± 4	81 ± 4	90 ± 5	57 ± 2	82 ± 4	98 ± 5	58 ± 4	81 ± 5	93 ± 6	55 ± 2	61 ± 3	62 ± 3
Phenylalanine	64 ± 3	80 ± 3	81 ± 2	59 ± 2	82 ± 3	93 ± 2	60 ± 3	85 ± 2	97 ± 3	60 ± 2	66 ± 2	66 ± 2
Tryptophan	50 ± 3	71 ± 4	76 ± 4	52 ± 2	49 ± 2	47 ± 3	46 ± 3	50 ± 3	48 ± 3	48 ± 2	50 ± 2	45 ± 3
Ornithine	55 ± 5	72 ± 5	77 ± 5	56 ± 5	89 ± 7	108 ± 7	54 ± 4	71 ± 4	79 ± 6	59 ± 8	63 ± 7	62 ± 7
Lysine	178 ± 10	323 ± 10	374 ± 10	175 ± 6	299 ± 12	348 ± 12	171 ± 8	174 ± 12	159 ± 10	166 ± 9	192 ± 8	200 ± 8
Histidine	80 ± 3	94 ± 4	93 ± 3	78 ± 4	93 ± 5	94 ± 4	80 ± 3	96 ± 3	98 ± 3	77 ± 4	81 ± 5	81 ± 4
Arginine	83 ± 5	107 ± 4	113 ± 4	82 ± 4	163 ± 11	196 ± 13	82 ± 4	103 ± 5	107 ± 6	83 ± 3	86 ± 3	83 ± 4

[1] $\bar{x}$ ± SEM; n = 8. For composition of drinks 5–8, *see* Tables 1 and 2.

free amino acids to a wheat protein hydrolysate in trials 9 and 10 clearly resulted in significantly higher plasma leucine and phenylalanine responses than did ingestion of the protein (hydrolysates) in drinks 5, 6, 7, and 8. In general, the ingestion of the intact protein resulted in less marked increases in various plasma amino acid concentrations within the 2-h period than did the ingestion of the protein hydrolysates. More detailed information is provided in Tables 3–6.

TABLE 5
Plasma amino acid concentrations after ingestion of drinks 9 and 10 containing amino acid or protein hydrolysate and carbohydrate[1]

	9 (leu/phe and wheat)			10 (leu/phe/arg and wheat)		
	0 min	60 min	120 min	0 min	60 min	120 min
			μmol/L			
Threonine	104 ± 6	102 ± 6	87 ± 6	107 ± 6	111 ± 8	99 ± 8
Serine	96 ± 8	99 ± 10	84 ± 9	99 ± 8	103 ± 9	89 ± 9
Asparagine	52 ± 2	47 ± 2	39 ± 3	53 ± 6	47 ± 3	39 ± 3
Glutamate	71 ± 11	62 ± 4	68 ± 7	56 ± 8	48 ± 4	42 ± 5
Glutamine	673 ± 12	702 ± 12	686 ± 29	702 ± 27	750 ± 26	740 ± 18
Proline	174 ± 22	207 ± 27	216 ± 21	193 ± 32	250 ± 32	257 ± 31
Glycine	239 ± 13	217 ± 12	197 ± 13	232 ± 11	216 ± 10	203 ± 13
Alanine	338 ± 21	346 ± 24	337 ± 20	316 ± 24	362 ± 18	369 ± 15
Citrulline	33 ± 2	37 ± 3	43 ± 3	33 ± 2	41 ± 3	49 ± 3
α-Aminobutyrate	15 ± 1	14 ± 1	11 ± 1	18 ± 2	17 ± 2	14 ± 2
Valine	241 ± 7	234 ± 5	180 ± 6	247 ± 10	245 ± 8	188 ± 7
Methionine	22 ± 1	21 ± 1	16 ± 1	23 ± 1	25 ± 1	19 ± 1
Isoleucine	68 ± 2	65 ± 2	38 ± 2	68 ± 4	70 ± 4	39 ± 2
Leucine	122 ± 4	518 ± 37	654 ± 43	122 ± 4	455 ± 21	542 ± 20
Tyrosine	54 ± 3	112 ± 9	141 ± 10	53 ± 2	114 ± 8	150 ± 13
Phenylalanine	61 ± 3	342 ± 34	526 ± 37	60 ± 1	270 ± 19	411 ± 19
Tryptophan	46 ± 2	42 ± 2	32 ± 2	43 ± 2	43 ± 2	35 ± 2
Ornithine	57 ± 5	65 ± 6	70 ± 6	58 ± 5	123 ± 8	175 ± 14
Lysine	169 ± 11	164 ± 12	147 ± 14	160 ± 7	177 ± 9	155 ± 11
Histidine	77 ± 2	76 ± 2	69 ± 2	78 ± 4	79 ± 4	70 ± 3
Arginine	83 ± 3	94 ± 4	95 ± 5	80 ± 3	289 ± 15	370 ± 20

[1] $\bar{x}$ ± SEM; n = 8. Leu, leucine; phe, phenylalanine; arg, arginine. For composition of drinks 9–10, *see* Tables 1 and 2.

TABLE 6
Plasma amino acid responses after ingestion of drinks containing carbohydrate and amino acid or protein hydrolysate[1]

	1 (control)	2 (arg)	3 (leu/phe/arg)	4 (leu/phe/arg/gln)	5 (whey)	6 (pea)	7 (wheat)	8 (casein)	9 (leu/phe and wheat)	10 (leu/phe/arg and wheat)
					mmol·2h·L^{-1}					
Threonine	−1.3 ± 0.3[2–5]	−1.0 ± 0.4[2–5]	−2.2 ± 0.2[2–6]	−1.9 ± 0.2[2–6]	10.2 ± 0.7[3–11]	5.4 ± 0.5[2,4–11]	2.5 ± 0.5[2,3,6–11]	1.2 ± 0.2[2,3,7–11]	−0.7 ± 0.2[2–5]	0.0 ± 0.2[2–4,9,10]
Serine	−1.2 ± 0.1[2–5]	−1.3 ± 0.3[2–5]	−2.0 ± 0.2[2–6,11]	−1.8 ± 0.2[2–6,11]	4.5 ± 0.4[5–11]	3.9 ± 0.3[5–11]	3.3 ± 0.4[5–11]	0.8 ± 0.2[2–4,7–10]	−0.1 ± 0.2[2–4,9,10]	−0.1 ± 0.1[2–4,9,10]
Asparagine	−0.7 ± 0.1[2–4]	−0.6 ± 0.2[2,3]	−1.2 ± 0.2[2–5]	−1.1 ± 0.1[2–5]	3.5 ± 0.4[4–11]	4.5 ± 0.4[6–11]	0.8 ± 0.2[2,3,6,7,9–11]	0.6 ± 0.1[2,3,9,10]	−0.7 ± 0.1[2–4]	−0.8 ± 0.6[2–4]
Glutamate	0.2 ± 1.1	−0.3 ± 1.0	−2.6 ± 1.1	−3.4 ± 1.1	0.3 ± 0.5	0.0 ± 0.5	−2.1 ± 0.7	−0.6 ± 0.6	−0.6 ± 0.9	−0.9 ± 0.6
Glutamine	−4.6 ± 2.2[2–4,6,10]	−1.6 ± 2.7[3,4,10]	0.7 ± 2.3[4]	6.8 ± 2.2[5,7,8]	5.3 ± 1.4[7]	5.7 ± 2.2[7,8]	11.8 ± 1.5[5–9,11]	−0.6 ± 1.9[4,10]	2.1 ± 1.3[4]	4.0 ± 1.3[4,7]
Proline	−1.4 ± 0.4[2–6,11]	0.6 ± 0.9[2–6,9,10]	−3.3 ± 0.7[2–6,8,11]	−3.3 ± 0.4[2–6,8,11]	9.4 ± 0.7[3–11]	5.5 ± 0.6[2,4,7–10]	15.1 ± 1.0[2,3,5–11]	4.0 ± 0.3[2,4,7–10]	3.2 ± 0.5[2,4,7,9,10]	5.3 ± 0.7[2,4,7–10]
Glycine	−1.7 ± 0.5[3,4,9,10]	−1.8 ± 0.6[3,4,9,10]	−5.1 ± 0.6[2–8,11]	−4.7 ± 0.5[2–8,11]	0.0 ± 1.0[2,4,6,9,10]	4.9 ± 0.6[2,5–11]	3.0 ± 0.6[2,5–11]	−0.9 ± 0.3[3,4,9,10]	−2.6 ± 0.4[3,4,9,10]	−1.8 ± 0.5[2–4,9,10]
Alanine	1.1 ± 1.3[2–4]	1.6 ± 1.9[2–4]	−3.2 ± 1.4[2–6]	−0.4 ± 1.4[2–4]	15.0 ± 1.6[5–11]	12.7 ± 1.3[5–11]	10.1 ± 1.4[5–11]	3.0 ± 1.7[2–4,9]	0.4 ± 1.0[2–4]	4.3 ± 1.2[2–4,9]
Citrulline	−1.2 ± 0.1[2–6,8–11]	−0.6 ± 0.1[4,6,7,9–11]	0.1 ± 0.1[4–8,10,11]	0.7 ± 0.1[2,3,5,7–9]	−0.3 ± 0.1[4,6,7,10,11]	−0.3 ± 0.1[4,6,7,10,11]	0.6 ± 0.2[2,3,5,7–9]	−0.5 ± 0.1[4,6–11]	0.6 ± 0.2[2,3,5,7–9]	1.0 ± 0.2[2,3,5,7–9]
α-Aminobutyrate	−0.1 ± 0.1[2]	-0.2 ± 0.1[2]	−0.2 ± 0.2[2]	−0.3 ± 0.1[2]	0.4 ± 0.2[6–11]	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	−0.2 ± 0.0[2]	−0.2 ± 0.0[2]
Valine	−3.4 ± 0.4[2–5,9,10]	−3.4 ± 0.4[2–5,9,10]	−6.1 ± 0.5[2–8,11]	−5.7 ± 0.4[2–8,11]	11.2 ± 0.7[3–11]	7.3 ± 0.6[2,4–11]	4.5 ± 0.4[2,3,5–11]	1.7 ± 0.3[2–4,6–11]	−2.3 ± 0.4[2–5,9,10]	−1.9 ± 0.5[2–5,9,10]
Methionine	−0.5 ± 0.1[2,4–6]	−0.4 ± 0.1[2,4,5]	−0.5 ± 0.1[2,4–6]	−0.6 ± 0.1[2,4–6,11]	1.6 ± 0.1[3–11]	−0.3 ± 0.1[2,4,5]	0.6 ± 0.1[2,3,6–11]	0.3 ± 0.1[2,3,7–11]	−0.2 ± 0.1[2,4,5,10]	0.0 ± 0.1[2,4,7,9,10]
Isoleucine	−1.9 ± 0.2[2–6]	−2.2 ± 0.2[2–6]	−2.8 ± 0.2[2–6,11]	−2.9 ± 0.1[2–6,11]	8.7 ± 0.4[3–11]	4.4 ± 0.4[2,4–11]	2.5 ± 0.3[2,3,5–11]	0.7 ± 0.1[2–4,6–11]	−1.1 ± 0.1[2–5,9,10]	−0.8 ± 0.2[2–5,7–10]
Leucine	−3.4 ± 0.3[2–4,6,9–11]	−3.5 ± 0.4[2–4,6,9–11]	47.7 ± 3.7[2–8,10,11]	37.1 ± 3.1[2–5,7–9]	12.2 ± 0.6[5–11]	6.9 ± 0.7[6–11]	5.3 ± 0.5[6–11]	0.8 ± 0.2[2,6,9–11]	39.7 ± 3.4[2–5,7–9]	32.6 ± 1.7[2–5,7–9]
Tyrosine	−0.8 ± 0.2[2–4,6,9–11]	−1.1 ± 0.2[2–4,6,9–11]	4.9 ± 0.7[5,7,8]	6.3 ± 1.5[2–5,7,8]	2.3 ± 0.2[6–8,10,11]	2.8 ± 0.3[6–8,10,11]	2.4 ± 0.5[6–8,10,11]	0.6 ± 0.2[6,9–11]	6.1 ± 0.8[2–5,7,8]	6.6 ± 0.8[2–5,7,8]
Phenylalanine	−1.1 ± 0.1[6,9–11]	−1.3 ± 0.3[6,9–11]	39.4 ± 2.3[2–8,10,11]	28.5 ± 2.7[2–5,7–9]	1.5 ± 0.1[6,9–11]	2.4 ± 0.2[6,9–11]	2.6 ± 0.1[6,9–11]	0.5 ± 0.2[6,9–11]	30.8 ± 3.2[2–9]	23.1 ± 1.6[2–5,7–9,11]
Tryptophan	−0.6 ± 0.1[2]	−0.1 ± 0.1	−0.9 ± 0.1[2,4]	−0.3 ± 0.6[2]	2.0 ± 0.2[3–7,9–11]	−0.3 ± 0.2[2]	0.2 ± 0.1[2,9]	0.1 ± 0.1[2]	-0.6 ± 0.1[2]	−0.2 ± 0.2[2]
Ornithine	−0.6 ± 0.1[2–4,6,8–10]	7.7 ± 0.6[2–5,7,9,11]	11.6 ± 0.9[2–8,10,11]	8.9 ± 0.8[2–5,7,9,11]	1.7 ± 0.1[3,6–10]	3.5 ± 0.3[2,5–11]	1.8 ± 0.2[6–10]	0.4 ± 0.2[3,6,8–10]	0.9 ± 0.2[3,6,8–10]	7.4 ± 0.5[2–5,7,9,11]
Lysine	−1.4 ± 0.4[2,3,5,9]	0.0 ± 0.6[2,3,5,9]	1.1 ± 0.6[2,3,7,8]	−0.2 ± 0.6[2,3,5]	14.5 ± 0.5[4–11]	12.6 ± 0.9[4–11]	-0.2 ± 0.5[2,3,5]	2.6 ± 0.2[2–4,7,8,10,11]	−1.0 ± 0.2[2,3,5]	0.9 ± 0.3[2,3]
Histidine	−0.7 ± 0.[2–5,9]	−0.7 ± 0.2[2–5]	−1.4 ± 0.2[2–7,11]	−1.1 ± 0.1[2–6,11]	1.2 ± 0.2[5–11]	1.4 ± 0.2[5–11]	1.6 ± 0.2[5–11]	0.4 ± 0.1[2–4,7–11]	−0.3 ± 0.1[2–5,9,10]	−0.2 ± 0.1[2–4,9,10]
Arginine	−1.2 ± 0.2[3,6,8–10]	16.5 ± 0.8[2–7,9–11]	31.3 ± 1.9[2–8,10,11]	23.5 ± 1.5[2–5,7,9,11]	2.3 ± 0.3[3,6,8–10]	8.2 ± 0.8[2,4–11]	2.0 ± 0.4[3,6,8–10]	0.2 ± 0.1[3,6,8–10]	1.0 ± 0.3[3,6,8–10]	21.2 ± 1.4[2–5,7,9,11]

[1] Plasma amino acid response expressed as area under the curve minus baseline values. $\bar{x}$ ± SEM; n = 8. For composition of drinks 1–10, *see* Tables 1 and 2. Arg, arginine; leu, leucine; phe, phenalalanine; gln, glutamine.

[2] Significantly different from drink 5, $P < 0.05$.

[3] Significantly different from drink 6, $P < 0.05$.

[4] Significantly different from drink 7, $P < 0.05$.

[5] Significantly different from drink 8, $P < 0.05$.

[6] Significantly different from drink 10, $P < 0.05$.

[7] Significantly different from drink 1, $P < 0.05$.

[8] Significantly different from drink 2, $P < 0.05$.

[9] Significantly different from drink 3, $P < 0.05$.

[10] Significantly different from drink 4, $P < 0.05$.

[11] Significantly different from drink 9, $P < 0.05$.

TABLE 7
Statistical analysis of questionnaire results[1]

	Test drink no.									
Question	1	2	3	4	5	6	7	8	9	10
1 (nausea)	—	—	—	—	—	—	—	—	—	—
2 (bloated feeling)	4	—	—	—	4	—	4	6	—	4
3 (belching)	3	3	—	—	3	—	3	3,4	—	3
4 (gastrointestinal cramps)	—	—	—	—	—	—	—	—	—	—
5 (vomiting)	—	—	—	—	—	—	—	—	—	—
6 (diarrhea)	2	—	2	2	2	2	2	2	2	2
7 (urge to defecate)	2	—	2	2	2	2	2	2	2	2
8 (urge to urinate)	—	—	—	—	—	—	—	—	—	—
9 (headache)	4	—	—	—	—	4	4	4	4	4
10 (dizziness)	—	—	—	—	—	—	—	—	—	—

[1] The numbers within the table represent the drinks with a significantly ($P < 0.05$) higher score for the indicated symptom compared with the drink indicated in the column head.

Strong positive correlations were found between insulin responses and increases in plasma leucine ($P < 0.003$), phenylalanine ($P < 0.02$), tyrosine ($P < 0.0001$), and citrulline ($P < 0.0023$). A significant negative correlation was observed between insulin responses and plasma glutamate ($P < 0.02$) concentrations.

The data derived from the questionnaires were analyzed, and significant differences between drinks are indicated in **Table 7**. Gastrointestinal problems were found after administration of the arginine drink (2). Subjects scored higher for presence of urge to defecate and diarrhea (3.1 and 3.4, respectively) after ingestion of drink 2 than after ingestion of all other drinks (1.2 and 1.0, respectively). Eventually, all subjects experienced severe diarrhea for several hours during and after ingestion of test drink 2 (0.4 g arginine kg body $wt^{-1} \cdot h^{-1}$). This was not observed in trials 3 and 4, in which dosages of 0.13 and 0.10 g arginine $\cdot$ kg body $wt^{-1} \cdot h^{-1}$, respectively, were ingested. Furthermore, significantly higher scores for the presence of headache and bloated feeling were reported after ingestion of the free amino acids in drink 4 (1.6 and 3.3, respectively) than after ingestion of several other test drinks (mean overall score: 1.1 and 1.7, respectively). In addition, a significantly higher score for belching was found after ingestion of the free amino acids in drink 3 (score: 3.4) than after ingestion of several other test drinks (mean overall score: 1.8). These symptoms were absent in the trials that combined those free amino acids with a wheat protein hydrolysate (Table 7).

DISCUSSION

The results of this study indicate that oral ingestion of some amino acid mixtures in combination with carbohydrates can produce strong insulinotropic effects. Four free amino acids (leucine, phenylalanine, arginine, and glutamine) were tested. Several in vitro studies showed that these amino acids have a strong stimulating effect on insulin release by pancreatic β-cells (12–14, 19). Floyd et al (5, 7) observed that 30 g arginine injected intravenously in vivo in human subjects led to an equal insulin response as occurred with the mixture of 10 amino acids (30 g in total; arginine, lysine, phenylalanine, leucine, valine, methionine, histidine, isoleucine, threonine, and tryptophan).

The data in this study show clearly that oral ingestion of large amounts of free arginine (0.4 g arginine $\cdot$ kg body $wt^{-1} \cdot h^{-1}$, as ingested in trial 2) is not an effective means of increasing plasma insulin concentrations (Figure 4A) and plasma arginine concentrations (Table 3). Ingestion of drink 2 caused severe diarrhea and the urge to defecate in all subjects for several hours during and after the trial. These gastrointestinal problems appeared to prevent intestinal absorption of the arginine because lower concentrations of arginine were seen in plasma after ingestion of drink 2 than after ingestion of drinks 3, 4, and 10 (ingestion rates of 0.13, 0.10, and 0.07 g arginine $\cdot$ kg body $wt^{-1} \cdot h^{-1}$, respectively). These problems also indicate that in sports practice, ingestion of large amounts of arginine to stimulate growth hormone release and muscle anabolism is not recommended. On the other hand, low doses of arginine (<2 g), as present in commercial sports supplements, do not increase plasma insulin and growth hormone concentrations (23–25).

In later studies, Floyd et al (8, 9) investigated the combined effect of intravenous administration of glucose with combinations of amino acids and found that arginine-leucine and arginine-phenylalanine resulted in the strongest increase in plasma insulin concentrations. We investigated the insulinotropic effect of oral administration of a mixture of arginine, leucine, and phenylalanine (drink 3). A significantly greater insulin response was seen compared with the control trial (101% greater: $P < 0.05$). The increased plasma insulin concentration was attended by a strong significant increase in plasma arginine, leucine, and phenylalanine concentrations (Tables 3 and 6).

Sener and Malaisse (16) observed that the addition of leucine to the incubation medium stimulates insulin release by pancreatic β-cells in vitro. The mechanism behind this effect was investigated, and it was found that leucine activates glutamate dehydrogenase activity in pancreatic β-cells. This subsequently leads to an increase in tricarboxylic acid cycle activity and oxygen consumption of the pancreatic β-cells and is attended by increased insulin production. The addition of glutamine to the incubation medium potentiates the leucine-induced increase in insulin release by providing substrate for glutamate dehydrogenase, whereas glutamine per se has no effect (16). Consequently, we studied the effect of the addition of glutamine to drink 4. However, we observed no differences in insulin response between trials 3 and 4, suggesting that in humans in vivo, enough glutamine is present (600–800 $\mu mol \cdot L^{-1}$ in plasma; Table 3) to serve as fuel for the pancreas. Also note that the addition of free glutamine hardly influenced plasma glutamine concentrations. Plasma glutamine responses after the ingestion of drink 4 were in fact not significantly different than after ingestion of most other drinks (drinks 3, 5–7, 9, and 10; Tables 4 and 5). No significant differences were found between

the insulin responses in test trials 5, 6, and 7 and the carbohydrate-only trial. Nonetheless, mean insulin responses were 55%, 25%, and 81% greater, respectively, than those observed in the control trial. There were no differences in plasma leucine and phenylalanine responses between the different protein hydrolysates tested (Tables 4 and 6). None of the hydrolysates was associated with gastrointestinal or other complaints.

To compare the insulinotropic effect of the ingestion of the protein hydrolysates with that of an intact protein, sodium-casein was provided in drink 8. This resulted in an insulin response that was not significantly different from that found with the control trial (30% greater) and tended to be less than the responses observed after ingestion of the protein hydrolysates (drink 5 and 7). After ingestion of the intact protein, plasma amino acid responses over this 2-h period were in general lower than the responses observed after ingestion of the protein hydrolysates (Table 6). We conclude that the use of protein hydrolysates is preferred to stimulate insulin secretion because this results in a faster increase in plasma amino acid concentrations during a 2-h period than does intact protein. Another practical disadvantage of the use of an intact protein when ingested as a drink is that most intact proteins have poor solubility in water.

On the basis of the results obtained after trials 1–8, it was concluded that ingestion of free glutamine is not required to obtain an optimal insulin response, whereas the use of free arginine should be restricted to low doses. It was further concluded that ingestion of relative large quantities of amino acids (arginine, leucine, phenylalanine, and glutamine) can cause gastrointestinal and other complaints (drinks 2, 3, and 4; Table 7). In an attempt to combine gastrointestinal tolerance and palatability with a maximal insulin response, drinks 9 and 10 were prepared. Ingestion of both drinks resulted in large insulin responses. In trial 9, leucine and phenylalanine were ingested in combination with the wheat protein hydrolysate and we observed a larger insulin response (103% greater, $P < 0.05$) than with the carbohydrate-only trial that was similar to the response found in trial 3 but without the occurrence of any gastrointestinal and other complaints. Plasma leucine and phenylalanine responses were higher than with the control, arginine, and protein (hydrolysate) drinks but were lower than the response after the ingestion of drink 3. In trial 10, free arginine was added to this mixture, but this showed no further increase in insulin response (69% greater than with the control trial).

Regression analysis of the insulin responses and the changes in plasma amino acid concentrations over the 2-h period showed a strong positive correlation between the observed insulin response and changes in plasma leucine ($P < 0.003$), phenylalanine ($P < 0.02$), and tyrosine ($P < 0.0001$) concentrations. This agrees with several in vitro studies in which β-cells of the pancreas were incubated with leucine and phenylalanine (11–20) and with the in vivo studies by Floyd et al (5–10) in which amino acids were infused. The correlation observed with tyrosine concentrations may be explained by the fact that tyrosine is formed by the hydroxylation of phenylalanine when large amounts of phenylalanine are ingested (26). As such, tyrosine concentrations were higher in drinks containing large amounts of phenylalanine (Tables 3–5). In addition, we observed an unexplained positive correlation with citrulline ($P < 0.002$) and a negative correlation with glutamate ($P < 0.019$).

The main conclusion is that oral intake of amino acids in combination with carbohydrates can result in an insulinotropic effect as much as 100% greater than with the intake of carbohydrates only. It was shown that a mixture of free leucine, phenylalanine, and arginine can produce a large insulinotropic effect when ingested in combination with carbohydrates. It was also shown that the addition of leucine and phenylalanine to a (wheat) protein hydrolysate can create a similar insulinotropic effect without any gastrointestinal discomfort. These mixtures should provide a useful tool to strongly elevate plasma insulin concentrations in future metabolic studies in healthy subjects and in patients.

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